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**D Muthia[1,4], E B Laconi[2], M Ridla[2], A Jayanegara[2], R Ridwan[3], R Fidriyanto[1,3], M Abdelbagi[1] and H Ramdani[1]**

[1]Graduate School of Animal Nutrition Sciences Faculty of Animal Sciences, IPB University, Jl. Agatis Kampus IPB Dramaga Bogor Indonesia, 16680.
[2]Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Jl. Agatis Kampus IPB Dramaga Bogor Indonesia, 16680.
[3]Research Center for Biotechnology-LIPI, Jl. Raya Bogor KM 46 Cibinong 16911, Indonesia
[4]Program Studi Budidaya Ternak, Politeknik Pertanian Negeri Payakumbuh, Jl. Raya Negara Tanjung Pati km.7 Harau, Sumatera Barat Indonesia, 26271.

E-mail: muthiadewi@gmail.com

**Abstract**. The use of rice straw for ruminant livestock has been restricted by the high fiber and low digestibility. This study aimed to evaluate the nutritional value and *in vitro* digestibility of rice straw processed by combining autoclave and ammoniation. The combination was performed to produce rice straw, which was able to digest by the rumen microorganism. The autoclave was conducted at 150°C, 1.2 atm, 60 minutes, whereas ammoniation used 3% urea. The study used a completely randomized design with four treatments i.e., untreated rice straw (A), rice straw + 3% urea (B), rice straw + autoclave (C), and rice straw + 3% urea + ensiling (D). Results revealed that urea addition increased ($P<0.05$) protein content of rice straw compared to the non-added urea. The pH of all sample treatments over 48 hours of *in vitro* analyses showed no significant difference, which means it with suit condition for the microorganism in the rumen (6.76– 6.82 ). The combination of rice straw + urea (B) showed higher ($P<0.05$) gas production in 24 and 48 hours in comparison to other treatments as well as increased ($P<0.05$). In conclusion, combining autoclave and ammoniation enhances the nutritional value and *in vitro* digestibility of rice straw.

## 1. Introduction

Rice straw, as agriculture's byproduct, has been used as an alternative feed for ruminants because it is abundant and available throughout the year in almost all Indonesia areas. Rice straw has been traditionally used as animal feed in Indonesia mainly. However, rice straw has high fiber characteristics, especially the lignocellulose component, which is resistant to digestive enzymes and low in protein. Cellulose is the main substance found in plant cell walls and helps the plant to remain stiff and strong and is the most abundant of all naturally occurring organic compounds. The characteristics of rice straw caused limiting factors in efforts to increase the productivity of ruminants in producing meat and milk [1].

Furthermore, Sarnklong et al (2010) explained that rice straw is characterized by a low degradation rate in the rumen, low rate of passage, and reduced feed intake [2]. Treatment techniques that have been widely used, namely are physical, chemical, and biological treatments, have been applied to

improve the nutritional quality of rice straw; these include chopping, grinding, alkali treatments (sodium hydroxide, calcium hydroxide), ammoniation or urea treatment, the addition of fiber degrading enzymes and inoculation with white-rot fungal species [2]. Regarding urea treatment of rice straw as feed, it has been repeatedly shown that use at an additional rate of 2–6% DM can increase the digestibility of rice straw by 2–100% compared to untreated rice straw [3], Jayanegara et al [1] and also increase in livestock productivity [4]. On the other hand, there are still few studies that use pressure heating in the high fiber feed on the treatment process. Pressure heating is usually used in the hydrolysis process. So far, farmers do not apply the already developed and recommended methods for improving rice straw utilization, i.e., physical, socio-economic conditions, and practical reasons. A drawback of urea treatment is that it needs more time, up to 3–7 weeks of treatment, before the rice straw can be fed to the animals [5]. This study aimed to evaluate the nutritional value and in vitro digestibility of rice straw processed by combining autoclave and ammoniation

## 2. Materials and methods

### *2.1. Sample preparation and treatment*

Rice straw was collected shortly after harvesting from paddy rice (Sungkam variety) field around Payobasuang, Payakumbuh District, West Sumatra. Rice straw was manually chopped (approximately 3 cm length), oven-dried at 60$^{o}$C for 24 h, and ground to pass a 1 mm screen using a hammer mill. The ground rice straw sample (500g each) was subjected to the following ammoniation with urea ($CO(NH_2)_2$) treatments:

A: untreated rice straw (control);
B: A + 3% urea (1 hour incubation);
C: A + autoclave (1.2 atm, 150$^{o}$C, 60 minutes);
D: A + 3% urea + autoclave

The autoclave was used to generate high temperature and pressure, i.e., 150$^{o}$C, 1.2 atm, 60 minutes, respectively. Urea was solubilized in water at 1:30 kg/l (equal to 3% v/v) prior to addition. Urea solution was sprayed to the ground rice straw (±60% DM) to ensure a homogenous mixture between both materials and incubated for one hour. Each treatment was conducted in four replicates.

### *2.2. Crude protein analysis*

Crude protein was determined by subtracting the moisture, fat, protein, and ash contents [6]. An amount of 0.3 g sample was weighed into a nitrogen-free filter paper (Whatman No. 41), folded, and transferred into a Kjeldahl flask (Kjeltec System 1002, Tecator). Blank was prepared with the only nitrogen-free paper being placed in the extraction apparatus. An amount of 5 mg cupric sulphate ($CuSO_4$) and 2 ml of concentrated sulphuric acid ($H_2SO_4$) 98% (v/v) was added. The Kjeldahl flask was then placed in a digestion block with increasing temperature to 550$^{o}$C until the whole sample became a clear solution. The sample solution was transferred to a distiller with a small amount of distilled water. When sodium hydroxide (NaOH 50% (w/v) was added, the solution in the distiller was turning blue. The distillation process was conducted until the 2% boric acid solution turning green color. The distillate was titrated with 0.01M hydrochloric acid. Titration volume was recorded when the color of distillate has changed from green to pink. Protein content was determined by multiplying the nitrogen percentage of the sample with a factor of 6.25. The crude protein content was calculated by the following formula:

$$\text{Percentage of Nitrogen (\%N)} = \frac{(\text{mL HCl sample- mL HCl blank}) \times \text{Molarity HCl} \times 14}{\text{Sample weight (mg)}} \times 100 \quad (1)$$

$$\text{Percentage of crude protein content} = \%\ \text{Nitrogen} \times 6.25\ (\text{factor}) \quad (2)$$

*2.3. In vitro rumen fermentation*

In vitro rumen fermentation was performed according to Theodorou et al [7]. Allocation of treatments into in vitro experimental units were following a randomized complete block design. Rumen inoculum was obtained from two fistulated Ongole Grade cattle at Research Center for Biotechnology, Indonesian Institute of Sciences (Lembaga Ilmu Pengetahuan Indonesia, LIPI), Cibinong, Bogor. Incubation was conducted in three replicates, and each treatment per run was represented by two fermentation tubes. Each run, an amount of 1 liter of McDougall buffer was prepared by mixing distilled water with the following chemicals: 9.8 g $NaHCO_3$, 4.63 g $Na_2HPO_4.2H_2O$, 0.57 g KCl, 0.47 g NaCl, 0.118 g $MgSO_4.7H_2O$, and 0.053 g $CaCl_2.2H_2O$. The buffer was then flushed with $CO_2$ to reach a final pH of 7.05 [8]. An amount of 0.5 g sample was inserted into a 50 ml fermentation tube made from polyethylene. The tube was subsequently added with 40 ml McDougall buffer solution and 10 ml rumen fluid and closed with a ventilated rubber cap. Fermentation was carried out in a shaker water bath maintained at 39$^o$C for 48 h. Fermentation was stopped by adding 2 drops of $HgCl_2$ and then centrifuged at 4,000 rpm, 4$^o$C for 10 min. The supernatant was taken for pH. The residue was added to 50 ml of 0.2% pepsin-HCl solution, incubated for another 48 h, and filtered through Whatman paper no. 41 under vacuum. It was oven-dried at 105$^o$C for 8 h and ashed in a furnace at 600$^o$C for 3 h to obtain DM and OM values of the residue, respectively. In vitro dry matter digestibility (IVDMD) and in vitro organic matter (IVOMD) were determined by subtracting DM and OM amounts in the residue, respectively, to their initial amounts before incubation. Two tubes per run with rumen fluid and buffer only but without sample substrate (blank) were incubated as described above, served as a correction factor to the DM and OM contents of the residuals [9,10].

*2.4. Statistical analysis*

The data obtained from experiments were analyzed using one way ANOVA. The results were expressed as mean values ± S.D (standard deviation), according to the following statistical model for completely randomized design:

$$Yij = \mu + \tau i + \varepsilon ij \quad (3)$$

Yij = The observed value
μ = The overall mean
Ti = The effect of different treatments
Eij = The random residual error

The significant effects of the treatment on a particular parameter were stated at the probability level of $P<0.05$. Duncan's multiple range test was employed as the posthoc test when the ANOVA result of a parameter showed a significant difference among treatments. Statistical analysis was conducted by using SPSS software version 22.0.

## 3. Results and discussion

According to the results (figure 1) shows a significantly different ($P<0.05$) between treatments. The increase in crude protein value in treatment was not only caused by the addition of urea (sample B) but also the effect of autoclave (sample C) and the combination of urea and autoclave (sample D), which caused an increase in crude protein.

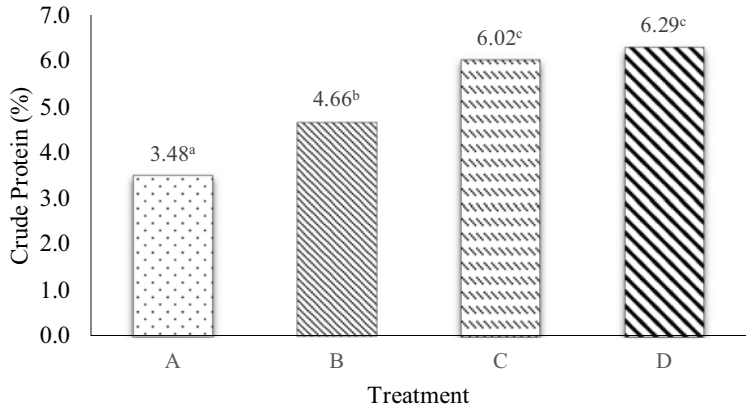


**Figure 1**. The crude protein content of rice straw on various treatments. A: untreated rice straw (control); B: A + 3% urea (1 hour incubation); C: A + autoclave (1.2 atm, 150°C, 60 minutes); D: A + 3% urea + autoclave.

Treatment with pressure and temperature by autoclave (1.2 atm, 150°C, 60 minutes) increased the protein value of rice straw. According to Gunun et al (2013), ammoniation with urea treatment is among the ammoniation technique in which the compound may release ammonia after being dissolved in water [4]. Although ammonia is less than NaOH to break down lignocellulose, thus less efficient in degrading fiber, but it provides nitrogen, which in turn may be converted to microbial protein and contributes to protein supply for animal production.

The pH values, gas production for 24 hours and 48 hours by treatments are presented in table 2. Treatment of autoclave, ammoniation, and their combination did not significantly affect pH ($P > 0.05$) but had a significant effect on gas production in vitro ($P<0.05$). There was a decrease in pH during the in vitro incubation for 48 hours, from the initial pH of 7.05. Rice straw with treatment (autoclave, ammoniation with urea) can break down fiber linkage, causing microorganism easer uses it. According to Ridwan et al (2018), the breakdown of readily fermentable materials can lead to critical changes in rumen conditions, such as decreasing pH and increase lactic acid levels, which contribute to metabolic acidosis [11].

**Table 1**. Mean of total pH and gas production of rice straw on various treatments

| Treatment | pH | Gas production on 24 h (mL) | Gas production on 48 h (mL) |
|---|---|---|---|
| A | $6.76 \pm 0.15^{a}$ | $27.87 \pm 1.70^{c}$ | $47.87 \pm 1.70^{b}$ |
| B | $6.76 \pm 0.03^{ab}$ | $33.75 \pm 3.17^{d}$ | $56.75 \pm 3.09^{c}$ |
| C | $6.82 \pm 0.026^{b}$ | $19.00 \pm 0.4^{a}$ | $40.25 \pm 0.28^{a}$ |
| D | $6.80 \pm 0.050^{ab}$ | $23.00 \pm 0.41^{b}$ | $47.62 \pm 0.63^{b}$ |
| SEM | 0.01018 | 1.48058 | 1.56225 |
| P-value | 0.088 | 0.000 | 0.000 |

A: untreated rice straw (control); B: A + 3% urea (1 hour incubation); C: A + autoclave (1.2 atm, 150°C, 60 minutes); D: A + B + C. Superscripts within the same column but different letters are significantly different at $P<0.05$.

The lowest gas production was in sample C (A + autoclave) and the highest was in sample B (rice straw + urea) at 24 hours in vitro. However, there was a change in the amount of gas after 48 hours in vitro that sample A (untreated rice straw) was not significantly different from sample D (A + urea + autoclave). There was an increase in the total of gas in the addition of urea (B), and a decrease in the

autoclave treatment (C) then increased again in the combination of autoclave and urea (D). This is because the rumen fluid from donor cattle contains microbes that use a different substrate from feed ingredients in the research (rice straw). High temperature and pressure induce the release of the acetyl groups from hemicellulose structure, increase the substrate's acidity, promote hemicellulose solubilization, and then increase the digestibility of rice straw [12]. Likewise, high temperature may induce a faster reaction between ammonia from urea and plant cell wall, resulting in a considerably shorter incubation period for a similar magnitude of digestibility improvement compared to control [1].

**Table 2**. Mean of total CH4 of rice straw on various treatments

| Treatment | $CH_4$ in 24 h | | $CH_4$ in 48 h | |
|---|---|---|---|---|
| | % | mL | % | mL |
| A | 1.75±0.86[a] | 0.49±0.26[a] | 4.25±0.28[a] | 2.03±0.14[a] |
| B | 3.62±0.25[b] | 1.22±0.04[b] | 5.12±0.47[ab] | 2.90±0.28[b] |
| C | 2.00±0.00[a] | 0.38±0.01[a] | 4.00±0.57[a] | 1.61±0.23[a] |
| D | 3.50±0.71[b] | 0.81±0.16[b] | 5.87±1.60[b] | 2.79±0.73[b] |
| SEM | 0.25400 | 0.09085 | 0.27717 | 0.16687 |
| P-value | 0.001 | 0.000 | 0.044 | 0.002 |

A: untreated rice straw (control); B: A + 3% urea (1 hour incubation); C: A + autoclave (150ºC, 1.2 atm, 60 min); D: A + B + C. Superscripts within the same column but different letters are significantly different (P<0.05). IVDMD: In vitro Dry Matter Digestibility.

Ammoniation (B) and combining ammoniation and autoclave (D) treatment showed significant differences in emission methane production of rice straw in comparison to those of the untreated rice straw or control on 24 h and 48 h (table 2). Methane gas ($CH_4$) is a byproduct of anaerobic fermentation of structural and non-structural carbohydrates by methanogens (methane-producing microbes) in the rumen. Methane gas formation in the rumen is the final result of feed fermentation that reduces $CO_2$ by $H_2$, which is catalyzed by enzymes from methanogenic microbes [13].

The lowest methane gas production was obtained in samples A (untreated rice straw) and C (A + autoclave), meaning that methanogenic bacteria in degrading fiber do not convert it into methane gas but are likely absorbed directly by the microbes so that the treatment reduces methane gas production. *In vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) shown in Table 3. Various treatments between processed rice straw showed significant differences (P<0.05) on IVDMD.

**Table 3**. Mean of in vitro and organic dry matter digestibility of rice straw on various treatments

| Treatment | IVDMD (%) | IVOMD (%) |
|---|---|---|
| A | 40.17±0.95[a] | 58.05±1.17[a] |
| B | 45.53±1.50[b] | 48.95±5.75[a] |
| C | 40.44±0.85[a] | 52.63±9.84[a] |
| D | 43.90±1.37[b] | 53.17±1.81[a] |
| SEM | 0.64709 | 1.54346 |
| P-value | 0.000 | 0.228 |

A: untreated rice straw (control); B: A + 3% urea (1 hour incubation); C: A + autoclave (150ºC, 1.2 atm, 60 min); D: A + B + C. Superscripts within the same column but different letters are significantly different (P<0.05). IVDMD: In vitro Dry Matter Digestibility.

Sample A (untreated rice straw) and sample C (A + autoclave) obtained lower on IVDMD compared to sample B (A + ammoniation) and sample D (A + combination B, C). This might cause that ammoniation with 3% urea on rice straw can sever fiber bonding, especially lignocelluloses on rice straw; otherwise, autoclave processing has not been able to break down on lignocellulose. It is known that combining autoclave and ammoniation on sample D can increase the IVDMD of rice straw. According to Sarnklong et al (2010), several physical, chemical, and biological treatments have been applied to improve the nutritional quality of rice straw; these include chopping, grinding, alkali treatments (sodium hydroxide, calcium hydroxide), ammoniation or urea treatment, the addition of fiber degrading enzymes and inoculation with white-rot fungal species [2]. Likewise, by urea addition to rice straw processing as ruminant feed, it has been repeatedly shown that the treatment (at 2–6% DM addition level) increased rice straw digestibility by 2–100% of untreated rice straw [3]. So, the combination of ammonia treatment, 3% urea supplementation with an autoclave (wet heating) has improved the digestibility of rice straw.

## 4. Conclusion

Combining autoclave (1.2 atm, 150$^{o}$C, 60 min) and ammoniation (3% urea) treatment improved the crude protein content of the rice straw. It also affected the total gas and methane production on *in vitro* digestibility.

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